ERDC/TN APCRP-EA-17
January 2008

Experimental Effects of Lime Application on Aquatic Macrophytes: 3. Growth Response Versus Exposure Time

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PURPOSE: This research investigates the effects of exposure time to lime-induced high pH and inorganic carbon limitation on the growth, survivorship, and reproductive success of Sago Pondweed (*Stuckenia pectinatus*).

BACKGROUND: Lime ($CaCO_3$ and $Ca(OH)_2$) applications have been shown to be effective in both suppressing submersed macrophyte growth and changing species composition (Babin et al. 1992; Chambers et al. 2001; Prepas et al. 2001a, 2001b; James et al. 2005; James and Barko 2006). The mode of action is believed to be inorganic carbon limitation at lime-induced high pH. James and Barko (2006) found that lime-induced pH increases above 10.3 caused calcite precipitation and a decrease in bicarbonate alkalinity, resulting in the suppression of shoot and root biomass and tuber production of Sago Pondweed. pH levels exceeding 10.3 for long periods appeared to be caustic, causing tissue damage and pigmentation loss (due to high base content).The length of exposure to lime-induced inorganic carbon limitation appeared to be important in these experiments as plant biomass and tuber production rebounded and recovered from the stress at low (i.e., < 4 days) exposure times. The objectives of this study were to examine the effects of exposure time to lime-induced high pH on growth and tuber formation of Sago Pondweed in mesocosms.

METHODS: Commercially obtained propagules (Kester's W.F.G. Nurseries, Omro, Wisconsin) of Sago Pondweed (*Stuckenia pectinatus* (L.) Boerner) were germinated in the laboratory on 18 June 2004. Sprouted plants were transplanted into polyethylene containers (10 cm wide × 10 cm deep × 16 cm height) filled with homogenized sediment (obtained from Eau Galle Reservoir, WI; see James et al. (2005) for a description of sediment characteristics) to a depth of 10 cm (one sprouted plant per container). Five replicate containers were planted for each of five separate lime exposure times, control conditions, and biomass at the time of lime exposure (total of 35 planted containers). The planted containers were transferred on 29 June to an outdoor clear fiberglass reference mesocosm (1.2 m dia. × 1.2 m height; 1400 L capacity) filled with local tap water (Ca = 57 mg • L^{-1}; total alkalinity = 200 mg Ca • L^{-1}; pH = 7.8; Conductivity = 422 µS; Mg = 28 mg • L^{-1}; NO_2NO_3-N = 0.2 mg • L^{-1}; K = 0.8 mg • L^{-1}; Na = 1.6 mg • L^{-1}; SO_4 = 21 mg • L^{-1}). Plants were allowed to grow for 21 days before exposure to lime-induced inorganic carbon limitation. Total alkalinity (138 mg L^{-1}) and pH (8.8) in this mesocosm at the time of planting differed from initial tap water conditions (see above) because plants from another experiment were grown in this tank prior to the start of the lime exposure experiment.

To another mesocosm containing local tap water and no planted containers, lime was applied as a slurry by mixing dry powder mass (as grams of $Ca(OH)_2$) with 8 L of tap water, then dispersed evenly over the surface of the mesocosm. Total alkalinity and pH at the time of lime application

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JAN 2008		**00-00-2008 to 00-00-2008**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Experimental Effects of Lime Application on Aquatic Macrophytes: 3. Growth Response Versus Exposure Time	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Information Technology Laboratory,U.S. Army Engineer Research and Development Center,3909 Halls Ferry Road,Vicksburg,MS,39180-6199**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**5**	

(17 July) were 174 mg L^{-1} and 8.4, respectively. Thus, a concentration dosage of 500 mg L^{-1} was chosen to reduce bicarbonate alkalinity to growth-limiting concentrations without increasing total alkalinity (see results) in this mesocosm.

On 19 July, plants grown in the reference mesocosm were transferred into the lime-treated experimental mesocosm (with the exception of control plants). At the time intervals listed in Table 1, five replicate containers each were removed from the treated mesocosm and transferred back to the control mesocosm for further growth. Thus, plant exposure to lime was inversely proportional to its exposure to control conditions during post-treatment. All planted containers were harvested on 13 September.

Table 1
Experimental treatments and lime application concentrations

Treatment	Exposure to lime-induced inorganic carbon limitation, days	Exposure to reference conditions after lime-induced inorganic carbon limitation, days
1	0	56
2	1	55
3	3	53
4	7	49
5	14	42
6	21	35

Table 2
Mean (± 1 standard error) pH and alkalinity species in the reference and experimental mesocosm during the 56-day study

Mesocosm	Mean pH	Total alkalinity, mg L^{-1}	Bicarbonate alkalinity, mg L^{-1}	Carbonate Alkalinity, mg L^{-1}	Hydroxide Alkalinity, mg L^{-1}
Experimental mesocosm	10.87 (0.02)	124.5 (9.8)	10.8 (1.02)	76.5 (7.8)	37.2 (1.7)
Reference mesocosm	9.2 (0.03)	105.7 (1.5)	89.8 (1.3)	15.0 (0.9)	0.9 (0.1)

Natural lighting during the experiment was controlled with a 30-percent shade cloth deployed 2 m above the surface of each mesocosm. Circulation pumps (Beckett Versa Gold G90AG; 0.34 m^3 min^{-1}) provided water circulation. Inorganic carbon chemistry was not altered by bubbling air into the mesocoms; thus equilibration between atmospheric and aqueous phases of CO_2 occurred via diffusional processes.

Shoot and root fresh and dry biomass were determined for each plant container at the end of the study. Five replicate planted containers were also sacrificed at the time of transfer to the treated mesocosm for determination of shoot and root biomass at the time of exposure to lime. Shoot material was briefly soaked in a 1 N hydrochloric acid solution to remove any calcium carbonate (calcite: $Ca(CO_3)$) deposits on the plant, gently rinsed in tap water, and dried at 90 ^oC for dry mass determination. Roots sieved from the sediment were dried to determine below-ground biomass (root material was not pretreated with 1 N HCl).

Throughout the study, in situ temperature, pH, dissolved oxygen, and conductivity were monitored in each mesocosm at 2- to 3-day intervals using a Hydrolab Surveyor 3 that was calibrated against known buffers and Winkler titrations. Integrated water column samples were collected to determine total alkalinity (expressed as mg $CaCO_3$ L^{-1}) as titration with 0.02 N sulfuric acid to an end-point of pH 4.5 (American Public Health Association (APHA) 1998). Free CO_2 and bicarbonate, carbonate, hydroxide alkalinity at 25 ^oC were estimated by calculation based on ionization constants (APHA 1998).

RESULTS AND DISCUSSION: Applying lime to the experimental mesocosm resulted in an increase in mean pH to ~ 10.9 and a decline in mean total and bicarbonate alkalinity from initial conditions (i.e., pH = 8.4; total alkalinity = 174 mg L^{-1}; bicarbonate alkalinity = 170 mg L^{-1}), indicating calcite precipitation and a reduction in inorganic carbon availability. However, hydroxide alkalinity accounted for 30 percent of the total alkalinity, indicating caustic basic conditions. Mean pH in the reference mesocosm was ~ 1.5 units lower than the experimental mesocosm throughout the study period. Although mean total alkalinity was lower, mean bicarbonate alkalinity was ~ 9 times in the reference mesocosm versus the experimental mesocosm.

All plants exposed to lime-induced inorganic carbon limitation exhibited stunted shoot and root biomass development compared to reference plants, even when exposed to lime treatment for less than a day (Figure 1). Plants exposed to lime treatment for ≤ 7 days exhibited minor to zero net shoot and root biomass increase over biomass levels at the time of lime exposure (Figure 1 and Table 3). For exposure times > than 7 days, a net loss in shoot and root biomass and a low or negative net growth rate was observed (Figure 1 and Table 3). In contrast, plants grown in the reference mesocosm exhibited shoot and root biomass development and a positive net growth rate during the study period. Tuber production occurred despite exposure to lime-induced growth inhibition; however, number produced per plant and tuber mass was significantly suppressed for lime exposure times exceeding 7 days.

These results lend support to the findings of James and Barko (2006) that exposure to lime-induced high pH can suppress the growth and reproduction of Sago pondweed. pH adjustment of the experimental mesocosm by lime application to greater than ~ 10.3 resulted in a decline in bicarbonate alkalinity below the growth-limiting target concentration of ~ 20 mg L^{-1} required to suppress Sago Pondweed growth without increasing total alkalinity found by James and Barko (2006). Exposure times to this growth-limiting concentration on the order of days resulted in significant suppression of shoot and root biomass and net growth and longer exposure times (on the order of 2 weeks) resulted in negative growth rates.

Similar to the findings of James and Barko (2006), tuber production was diminished at the longer exposure times of 14 and 21 days. However, plants did form tubers, even after the longer exposure times, which contrasted with the results of James and Barko. An important difference between this study and James and Barko was that lime-exposed plants were transferred back to a reference mesocosm that contained high bicarbonate alkalinity for further growth. This change in conditions provided the plants with an adequate inorganic carbon supply after the lime stress that was probably put into some tuber production. In the James and Barko study, bicarbonate alkalinity was allowed to slowly increase above the growth-limiting threshold after lime application, but it remained well

below reference levels throughout the post-lime treatment period and, thus, provided an additional stress that likely suppressed tuber production.

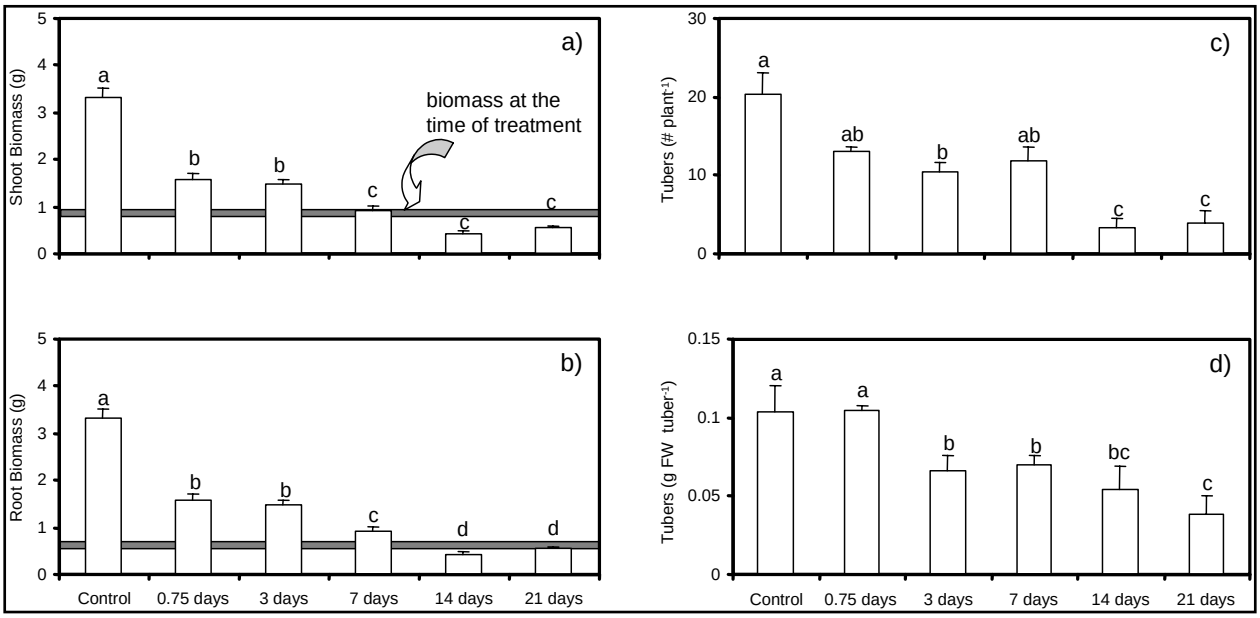

Figure 1. Variations in mean shoot biomass (a), root biomass (b), tuber production per plant (c), and tuber fresh mass (d) as a function of exposure (days) to a lime-induced increase in pH. Plants were grown for 31 days prior to exposure. After exposure, they were moved back to a reference tank containing untreated water. Vertical bars represent 1 standard error. Different letters indicate significant differences between means at the 5 percent level or less (ANOVA; Statistical Analysis System (SAS) 1994).

Table 3
Mean net shoot and root growth rate (± 1 standard error) over the 56 day period as a function of exposure time to lime-induced inorganic carbon limitation

Days of lime exposure (days)	Net shoot growth rate (mg DW d^{-1})	Net root growth rate (mg DW d^{-1})
0	23.6 (3.8) [a]	47.8 (3.4) [a]
0.75	5.3 (2.0) [b]	16.8 (2.1) [b]
3	2.7 (1.9) [b]	14.8 (1.5) [b]
7	-7.7 (1.7) [c]	4.9 (1.9) [c]
14	-11.1 (0.8) [c]	-4.1 (1.3) [d]
21	-9.0 (0.8) [c]	-1.6 (0.7) [d]

Because lime application resulted in a very high pH in the experimental mesocosm, caustic basic conditions probably played an important role in suppression of growth and reproduction, in addition to inorganic carbon limitation. Thus, dosage under field conditions should probably be targeted toward increasing the pH to 10.3 to induce calcite precipitation and inorganic carbon limitation without causing an increase in hydroxide alkalinity and basic conditions. More information is needed on growth response for lime dosages that raise the pH only to the carbonate-bicarbonate

equivalence point (~ pH 10.3) in order to better assess the effects of inorganic carbon limitation on growth suppression.

It appears from this research that exposure times to high pH need to be on the order of one to three weeks to suppress both shoot and root growth and the formation of tubers for Sago Pondweed. Other aquatic plants may differ in their growth responses to elevated pH and require longer or shorter periods of exposure. One application may not be enough to maintain elevated pH at critical growth-limiting levels over several days or weeks since inorganic carbon (and lower pH) will be gradually replenished through atmospheric reaeration and respiration processes. Two or multiple applications may be required to sustain high pH for the length of time required to suppress growth and tuber production. Field studies are needed to better evaluate dosage and exposure requirements in productive areas that exhibit diel swings in pH due to plant and microbial metabolism.

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This technical note should be cited as follows:

> James, W. F. 2007. *Experimental effects of lime application on aquatic macrophytes: 3. Growth response versus exposure time*. APCRP Technical Notes Collection. ERDC/TN APCRP-EA-17. Vicksburg, MS: U.S. Army Engineer Research and Development Center. *www.wes.army.mil/el/aqua*.

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